

The Mechanism of Nitrogen Fixation by Nitrogenase: The Next Stage

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S1. Analysis: To visualize the alternative dispositions of the two hydride bridges, we have built the bound hydrides onto the crystal structure of resting-state FeMo-co using Fe-H distances from model complexes,^{182,186} **Fig 5**.

Quantum chemical computations will test these alternatives. However, the experimentally determined relative orientation of the hyperfine tensors of the two hydrides provides a significant constraint on their placement within E₄. Given the stability of the FeMo-co structure that is likely imparted by the interstitial carbide, it seemed plausible to us that consideration of the constructed models of **Fig 5** would allow us to test the plausibility of these alternative proton distributions, even though it is beyond doubt that the structure of FeMo-co will distort upon substrate binding.

The equations developed to compute the anisotropic ¹H hyperfine interaction matrix of an [Fe-H-Fe] moiety place the small dipolar element, $t_2 \sim 0$, along the normal to the Fe-H-Fe plane.¹⁸¹ These equations further show that for the expected metrical parameters of $d_{\text{FeFe}} \sim 2.5 \text{ \AA}$ taken from the resting-state FeMo-co x-ray structure and an approximately symmetrical hydride, with $r_1 \sim r_2 = r \sim 1.6 \text{ \AA}$ as in a model compound¹⁸² (**Fig S1**), the existence of a rhombic dipolar tensor, \mathbf{T}_{rh} , requires $|K_1| \sim |K_2|$, with, the two large dipolar components rotated by $\gamma \sim \pi/4$, relative to the Fe-Fe vector, a geometry which orients the two large dipolar elements roughly along the Fe-H bonds, as can be seen from **Fig S1**; thus, for $\gamma = \pi/4$ and the chosen geometry, the dipolar components make an $\sim 5^\circ$ angle with the Fe-H bonds.

With this foundation, the experimentally determined relative orientation of the hyperfine tensors of the two hydrides provides a significant constraint on proposed pictures of E₄. It was shown that $t_2 \sim 0$ for one hydride is parallel to one of the large couplings for the other ($|t_1| \sim |t_3| = t$), and vice versa. The conclusion about tensor orientation is equivalent to a requirement that the normal to the [Fe-H-Fe] plane (the t_2 direction) of one hydride lies roughly along one of the two Fe-H bond of the other.

The models created for the four E₄ variant structures that have two hydrides bound to a common Fe₆, **Fig 5**, were tested to see if t_2 of the ‘2nd’ [Fe-H-Fe] (the normal to that plane) lies roughly parallel to either Fe-H bond of [Fe₆-H-Fe₇]; the resulting angles are given in **Table S1**. Two structures (**Fig 5, C & D**) are clearly incompatible with the experimentally determined relative orientation of the hyperfine interactions of the two hydrides, even recognizing the crudeness of this computation. The other two structures are notably, and comparably, more favorable.

TABLE S1

| Table S1 | |
|----------------------|--|
| Structure (Fig 5) | Lesser angle: $t_2(H_2)*Fe_{1,2}-H_1$ |
| A | 33^0 |
| B | 30^0 |
| C | 77^0 |
| D | 52^0 |

Figure S1. Visualization of a [Fe-H-Fe] fragment defining the metrical parameters that govern the anisotropic hyperfine interaction, $\mathbf{T} \sim [t, 0, -t]$, and showing the orientation of the hyperfine components. Adapted from ref ¹⁸¹.

FIGURE S2

